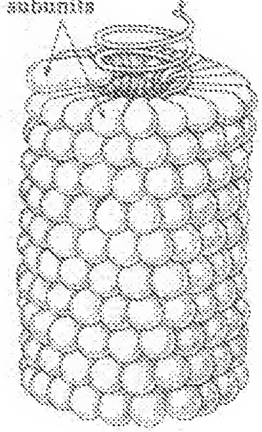
anywhere from two to twelve subunit chains in the smaller oligomeric proteins.

Since oligomeric proteins contain two or more polypeptide chains, usually not covalently attached to each other, it may appear improper or at least ambiguous to refer to such proteins as "molecules" and to speak of their "molecular weight." However, in most oligomeric proteins the separate chains are so tightly associated that the complete particle behaves in solution like a single molecule. Moreover, all the component subunits of oligomeric proteins are necessary for their biological function.

Figure 3-4
Portion of a tobacco mosaic virus particle, a supramolecular assembly containing 2,200 polypeptide chains and a molecule of RNA.



Supramolecular Assemblies of Proteins

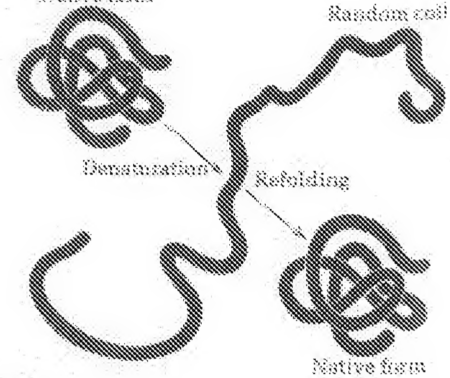
Sometimes a set of protein molecules functioning together occurs in cells as a cluster or complex that can be isolated in homogeneous or even crystalline form. An example of a cluster of functionally related macromolecules, called a *supramolecular assembly* or *complex*, is the *fatty acid synthetase complex*, which contains one molecule of each of the seven different enzymes required for the biosynthesis of fatty acids (page 660). This complex can be isolated from yeast cells in homogeneous form (Table 3-2). The largest supramolecular protein complexes are the *viruses*, complexes of proteins and nucleic acids; some viruses also contain lipids and metal ions. *Tobacco mosaic virus* (Figure 3-4), one of the smaller viruses, has a particle weight of nearly 40 million, of which about 5 percent, or 2 million, consists of ribonucleic acid. The remaining 38 million is contributed by the protein portion, consisting of some 2,200 identical polypeptide chains. However, virus particles behave like single homogeneous structures having a definite molecular weight because their subunit components stick together very tightly.

Denaturation

Most protein molecules retain their biological activity only within a very limited range of temperature and pH. Exposing soluble or globular proteins to extremes of pH or to high temperatures for only short periods causes most of them to undergo a physical change known as *denaturation*, in which the most visible effect is a decrease in solubility. Since no covalent bonds in the backbone of the polypeptide chain are broken during this relatively mild treatment, the primary structure remains intact. Most globular proteins undergo denaturation when heated above 60 to 70°C. Formation of an insoluble white coagulum when egg white is boiled is a common example of protein denaturation. But the most significant consequence of denaturation is that the protein usually loses its characteristic biological activity; e.g., heating usually destroys the catalytic ability of enzymes.

Denaturation is the unfolding of the characteristic native folded structure of the polypeptide chain of globular protein molecules (Figure 3-5). When thermal agitation causes the native folded structure to uncoil or unwind into a randomly looped chain, the protein loses its biological ac-

Figure 3-5
Denaturation and renaturation of a globular protein. After the polypeptide chain has been unfolded (by heating, by exposure to low pH, or by treatment with urea), it will often spontaneously refold to the native form.



tivity. Although each type of protein has an amino acid composition and sequence fixed during biosynthesis, the amino acid sequence as such does not *directly* endow a protein with its biological function or activity. However, we shall see that the amino acid sequence *ultimately* determines the biological activity of a protein because it determines the native conformation, or folded state, of the protein molecule, through interactions of the amino acid side chains with each other, with the solvent, and with other solutes. This conclusion follows from the discovery that denaturation, or unfolding, of native proteins into randomly coiled, biologically inactive forms is not irreversible, as was once thought. Many cases have now been observed in which an unfolded protein molecule spontaneously returns to its native biologically active form in the test tube, a process called *renaturation* (Figure 3-5). If the denatured protein was an enzyme, its catalytic activity returns on renaturation, without change in the specificity of the reaction catalyzed. However, renaturation of a denatured protein cannot evoke any biological activity that was not present in the original protein. These facts therefore indicate that the sequence of amino acids in the polypeptide chain contains the information required to specify its native folded conformation and that this native conformation determines its biological activity (Chapter 6).

The Functional Diversity of Proteins

Proteins have many different biological functions. Table 3-3 gives some representative types of proteins, classified according to function. The enzymes represent the largest class. Nearly 2,000 different kinds of enzymes are known, each catalyzing a different kind of chemical reaction. Enzymes have extraordinary catalytic power, far beyond that of man-made catalysts. They are highly specific in their function. The enzyme hexokinase catalyzes transfer of a phosphate group from adenosine triphosphate (ATP) to glucose, the first step in glucose metabolism. Other enzymes dehydrogenate fuel molecules. Still others, e.g., cytochrome c, transfer electrons toward molecular oxygen during respiration or, like DNA polymerase and amino acid–activating enzymes, participate in the biosynthesis of cell components. Each type of enzyme molecule contains an *active site*, to which its specific substrate is bound during the catalytic cycle. Many enzymes contain a single polypeptide chain; others contain two or more. Some enzymes, called *regulatory* or *allosteric* enzymes, are further specialized to serve a regulatory function in addition to their catalytic activity. Virtually all enzymes are globular proteins, as defined above. How enzymes catalyze chemical reactions is a major concern of modern biochemistry.

Another major class of proteins has the function of storing amino acids as nutrients and as building blocks for the growing embryo, e.g., *ovalbumin* of egg white, *casein* of milk, and *gliadin* of wheat seeds.

Some proteins have a transport function; they are capable of binding and transporting specific types of molecules via the blood. *Serum albumin* binds free fatty acids tightly and

Table 3-3 Classification of proteins by biological function

Type and examples	Occurrence or function
Enzymes	
Hexokinase	Phosphorylates glucose
Lactate dehydrogenase	Dehydrogenates lactate
Cytochrome c	Transfers electrons
DNA polymerase	Replicates and repairs DNA
Storage proteins	
Ovalbumin	Egg-white protein
Casein	A milk protein
Ferritin	Iron storage in spleen
Gliadin	Seed protein of wheat
Zein	Seed protein of corn
Transport proteins	
Hemoglobin	Transports O_2 in blood of vertebrates
Hemocyanin	Transports O_2 in blood of some invertebrates
Myoglobin	Transports O_2 in muscle cells
Serum albumin	Transports fatty acids in blood
β_1-Lipoprotein	Transports lipids in blood
Iron-binding globulin	Transports iron in blood
Ceruloplasmin	Transports copper in blood
Contractile proteins	
Myosin	Thick filaments in myofibril
Actin	Thin filaments in myofibril
Dynein	Cilia and flagella
Protective proteins in vertebrate blood	
Antibodies	Form complexes with foreign proteins
Complement	Complexes with some antigen-antibody systems
Fibrinogen	Precursor of fibrin in blood clotting
Thrombin	Component of clotting mechanism
Toxins	
Clostridium botulinum toxin	Causes bacterial food poisoning
Diphtheria toxin	Bacterial toxin
Snake venoms	Enzymes that hydrolyze phosphoglycerides
Ricin	Toxic protein of castor bean
Gossypin	Toxic protein of cottonseed
Hormones	
Insulin	Regulates glucose metabolism
Adrenocorticotrophic hormone	Regulates corticosteroid synthesis
Growth hormone	Stimulates growth of bones
Structural proteins	
Viral-coat proteins	Sheath around nucleic acid
Glycoproteins	Cell coats and walls
α-Keratin	Skin, feathers, nails, hoofs
Sclerotin	Exoskeletons of insects
Fibroin	Silk of cocoons, spider webs
Collagen	Fibrous connective tissue (tendons, bone, cartilage)
Elastin	Elastic connective tissue (ligaments)
Mucoproteins	Mucous secretions, synovial fluid

thus serves to transport these molecules between adipose tissue and other tissues or organs in vertebrates. The *lipoproteins* of blood plasma transport lipids between the intestine, liver, and adipose (fatty) tissues. *Hemoglobin* of vertebrate erythrocytes transports oxygen from the lungs to the tissues. Invertebrates have other types of oxygen-carrying protein molecules, such as the *hemocyanins*.

Other types of proteins function as essential elements in contractile and motile systems. *Actin* and *myosin* are the two major protein elements of the contractile system of skeletal muscle. Actin is a long, filamentous protein composed of many globular polypeptide chains arranged like a string of beads; myosin is a long rodlike molecule containing two helically intertwined polypeptide chains (Chapter 27). In muscles these proteins are arranged in parallel arrays and slide along each other during contraction.

Some proteins have a protective or defensive function. The blood proteins *thrombin* and *fibrinogen* participate in blood clotting and thus prevent the loss of blood from the vascular system of vertebrates, but the most important protective proteins are the *antibodies*, or *immune globulins*, which combine with and thus neutralize foreign proteins and other substances that happen to gain entrance into the blood or tissues of a given vertebrate. Indeed, the study of antibodies has led to the conclusion that each species of organism has its own specific set of protein molecules (page 112).

Toxins, i.e., substances that are extremely toxic to higher animals in very small amounts, represent another group of proteins and include *ricin* of the castor bean, *gossypin* of cottonseed, *diphtheria toxin*, and the toxin of the anaerobic bacterium *Clostridium botulinum*, which is responsible for some types of food poisoning.

Among the most interesting proteins are those functioning as hormones, such as growth hormone, or *somatotropin*, a hormone of the anterior pituitary gland. *Insulin*, secreted by certain specialized cells of the pancreas, is a hormone regulating glucose metabolism; its deficiency in man causes the disease diabetes mellitus.

Yet another class of proteins comprises those serving as structural elements. In vertebrates the fibrous protein *collagen* is the major extracellular structural protein in connective tissue and bone. Collagen fibrils, by forming a structural continuum, also help bind a group of cells together to form a tissue. Two other fibrous proteins in vertebrates are *elastin*, of yellow elastic tissue, and *α-keratin*, mentioned above. Cartilage contains not only collagen but also *glycoproteins*, which endow mucous secretions and synovial fluid in the joints of vertebrates with a slippery, lubricating quality.

Besides these major classes of proteins others have unusual functions. Spiders and silkworms secrete a thick solution of the protein *fibroin*, which quickly solidifies into an insoluble thread of exceptional tensile strength used to

form webs or cocoons. The blood of some fishes living in subzero Antarctic waters contains a protein that keeps the blood from freezing, aptly called "antifreeze" protein. Monellin is a sweet-tasting protein found in some fruits; when it is denatured, it no longer tastes sweet.

It is extraordinary that all proteins, including those having intense biological or toxic effects, are built from the same 20 amino acids, which by themselves have little or no biological activity or toxicity. Its three-dimensional conformation gives each type of protein its specific biological activity; its conformation is in turn determined by the specific sequence of the amino acids in its polypeptide chain(s) (Chapter 6).

Antibodies and the Immune Response; The Species Specificity of Proteins

Among the many different proteins in living organisms the *antibodies*, or *immune globulins*, have been of the utmost importance in demonstrating that proteins are specific for each species of organism. Antibody molecules appear in the blood serum and certain cells of a vertebrate in response to the introduction of a protein or some other macromolecule foreign to that species; such a species-foreign macromolecule is called the *antigen*. The specific antibody molecules generated in this manner can combine with the antigen which elicited their formation to form an *antigen-antibody complex* (Figure 3-6). This reaction, called the *immune response*, is the basis for the whole field of immunology. Immunity to a specific infectious disease can often be conferred by injecting very small amounts of certain macromolecular components (i.e., the antigenic components), of the causative microorganism or virus. A specific antibody or immune globulin is formed in response to the foreign antigen and may persist in the blood for a long time. If the causative microorganism should later gain access to the blood or lymph, these specific antibodies can inactivate or kill it by combining with its antigenic components. The immune response is given only by vertebrates and thus is a fairly recent product of biological evolution.

Antibody molecules have binding sites that are specific for and complementary to the structural features of the antigen that induced their formation. Usually the antibody molecule has two binding sites, making possible the formation of a three-dimensional lattice of alternating antigen and antibody molecules; since it ultimately precipitates from the serum, it is called the *precipitin* (Figure 3-6). The structure and origin of immune globulins are described in more detail in Chapter 35.

Antibodies are highly specific for the foreign proteins that evoke their formation. An antibody formed by a rabbit to injected hen's-egg albumin, for example, will combine with the latter but not with unrelated proteins such as human hemoglobin. Moreover, it is specific for the three-dimensional structure of native hen's-egg albumin, so that if the albumin is heated or denatured to unfold its polypeptide chains or is

Figure 3-6
The antigen-antibody reaction. The colored sites on the antigen are the determinants of antigenic specificity. The colored sites on the antibody molecule are structurally complementary to the determinant sites of the antigen. The antibody is divalent, but the antigen may be multivalent.

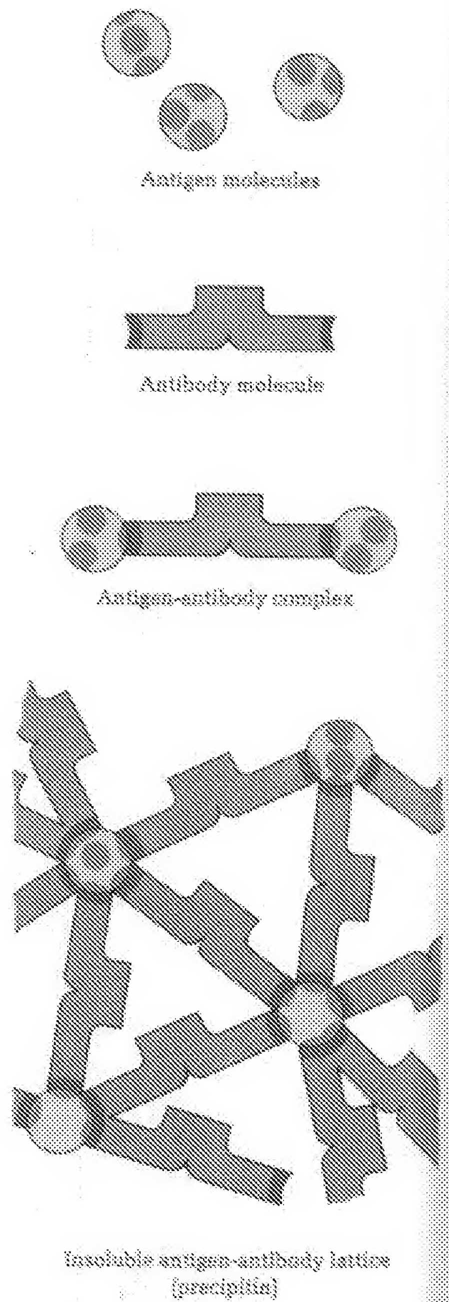


Table 3-4
albumin
with rabbi
serum alb

Species
Cow
Sheep
Pig
Cat
Horse
Man
Hamster
Rat
Dog
Mouse

† Data fro
manol., 86